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Research Note

Biological Studies of Filamentous Bacteria Associated with Cyathostomes from a Burchell's Zebra Hindgut

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ABSTRACT: Nematode–filamentous microbe association with cyathostomes collected from the hindgut of a Burchell's zebra was quantified. Cyathostomes were examined for the presence of filamentous bacteria with the aid of light and scanning electron microscopy. Sixty-seven percent of the females and 3% of the male nematodes were affected. The presence of filamentous bacteria was noted at or near the vulvar opening in the 5 most abundant cyathostome species. These species of cyathostomes from the most abundant to the least abundant were as follows: *Cyathostomum montgomeryi, Cylicocyclus triramosus, Cylicostephanus minutus, Cylicostephanus calicatus,* and *Cyathostomum tetracanthum.* In *C. montgomeryi,* bacteria colonized the anal orifice. The uterine tracts of the female cyathostomes were examined for the presence of eggs as a possible indication of the reproductive capacity, but the results were inconclusive. In 1 species, *C. triramosus,* the filamentous bacteria formed a dense mass that prevented thorough examination of the bacteria's position in possible penetration of the reproductive tract.

KEY WORDS: filamentous bacteria, cyathostomes, nematodes, zebra.

Large numbers of parasitic nematodes inhabit the hindguts of free-ranging zebras, and microbial communities have been reported in association with 2 of these nematode groups, the cyathostomes and atractids (Mackie et al., 1989; Krecek et al., 1992). Previous reports include ultrastructural studies of the components of these communities as well as proposed life cycles of 3 of the filamentous bacteria (Krecek et al., 1987; Els et al., 1991).

Cultivation of these microbial organisms has been attempted, with varying success (Mackie et al., 1989; Krecek et al., 1992). Currently, nematode material is obtained from the hindguts of zebras during postmortem examinations in various national parks in southern Africa. Not all populations of nematodes examined harbored these microbial communities. When microbes are present, the nematodes are stored in liquid nitrogen for further cultivation studies. The present study is the first to quantify the association of the filamentous bacteria and their cyathostome host species. The prevalence of female and male cyathostomes affected and the particular species of cyathostomes associated with the filamentous bacteria and the anatomical sites of colonization on these cyathostomes are described.

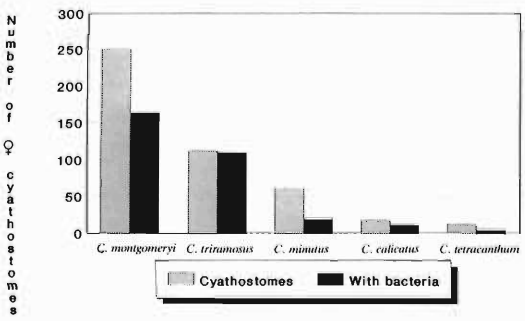


Figure 1. The number of females of the 5 most abundant cyathostome species associated with filamentous bacteria.

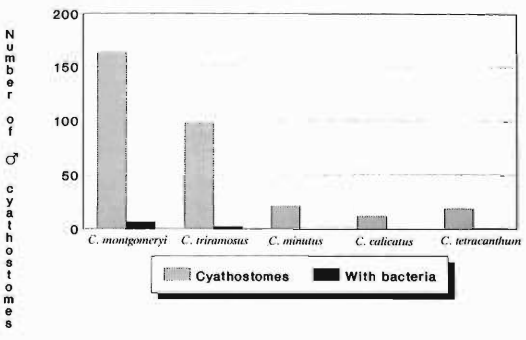


Figure 2. The number of males of the 5 most abundant cyathostome species associated with filamentous bacteria.

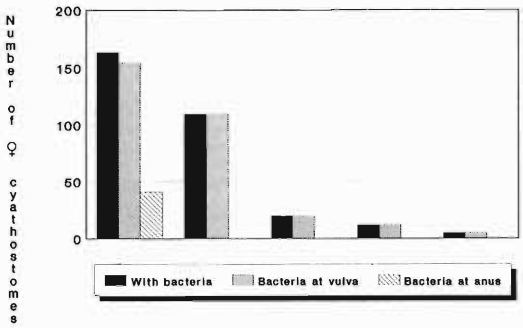
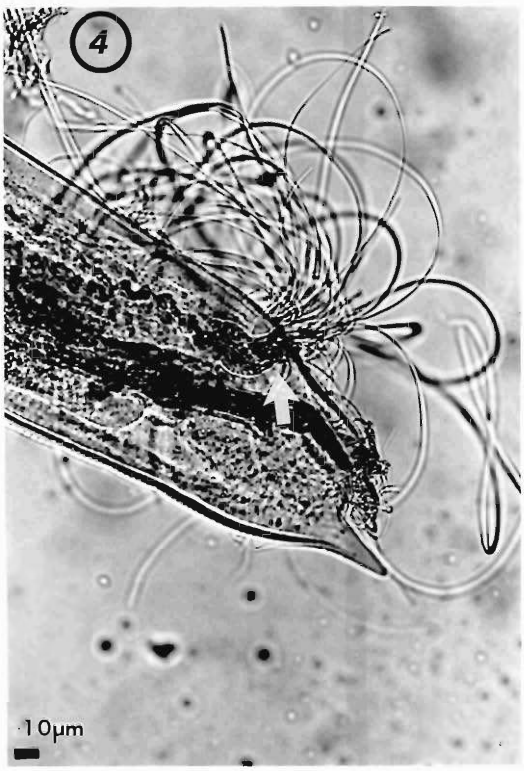
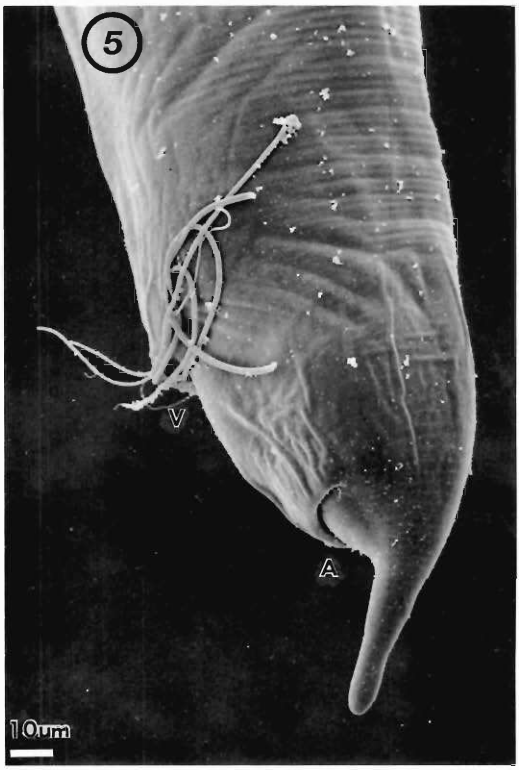


Figure 3. The number of female cyathostomes demonstrating filamentous bacteria at the vulvar and anal orifices.

A Burchell's zebra, *Equus burchelli antiquorum* H. Smith, 1841, from Etosha National Park, Namibia, was killed during August 1989 and processed at necropsy according to methods described previously (Malan et al., 1981; Els and Krecek, 1990). An aliquot of 100th of the ventral colon ingesta was examined, and the nematodes were recovered, mounted on glass slides in lactophenol, a clearing agent, and identified to species level (Theiler, 1923; Lichtenfels, 1975; Sciallo-Krecek, 1984). The attachment sites of filamentous bacteria to the cyathostomes were noted, especially to the oral, excretory, anal, and vulvar orifices. The orifices were examined for the presence of microbes both inside and outside these openings. The vulvar orifice was examined for the presence of eggs in the reproductive tract. For each cyathostome species, the number of nematodes affected at each orifice was counted. This quantification for each species and the number of male and female cyathostomes affected were recorded.

Abundance of each nematode species was derived by dividing the separate species by the total count of the aliquot, 877. For each species, 55 females were examined and the data extrapolated to the total. If there were fewer than 55 females all were examined and all of the males were examined. The chi-square test was used to compare sexes and species with sexes. The binomial test





Figures 4, 5. *Cyathostomum montgomeryi* female with filamentous bacteria on the posterior extremity. 4. Light microscopy shows a bacterial plug in vagina (arrow) with embedded microbes. 5. Scanning electron microscopy (SEM) with anus (A) and vulva (V).

was used when comparing the association of bacteria at the vulva and anus among species.

Whole cyathostomes were prepared for scanning and transmission electron microscopy by conventional techniques previously described (Els and Krecek, 1990).

The aliquot yielded 877 cyathostomes. Eight species were identified from the most to the least abundant as follows: *Cyathostomum montgomeryi* (Boulenger, 1920) K'ung, 1964, *Cylicocyclus triramosus* (Yorke and Macfie, 1920) Chaves, 1930, *Cylicostephanus minutus* (Yorke and Macfie, 1918) Cram, 1924, *Cylicostephanus calicatus* (Looss, 1900) Cram, 1924, *Cyathostomum tetracanthum* (Mehlis, 1831) Molin, 1861, in part, Looss, 1900, *Cylicodontophorus reineckei* Scialdo-Krecek and Malan, 1984, *Cylicostephanus longiconus* Scialdo-Krecek, 1983, and *Cylindropharynx* sp. The presence of filamentous bacteria was not noted on the latter 2 species, and only 1 of 3 specimens of *C. reineckei* demonstrated these bacteria. Therefore, these 3 species were not included in this study.

Among cyathostomes, 312 of 463 females examined and 11 of 414 males demonstrated an association with the microbes. The 5 most abundant cyathostome species in the population together with the proportion of females and males associated with the filamentous bacteria are given in Figures 1 and 2, respectively. Figure 3 indicates the presence of filamentous bacteria associated with the vulvar and anal orifices of the females of the 5 most abundant cyathostome species. Scanning electron and light microscopy demonstrate the presence of filamentous bacteria on females of *C. montgomeryi* and *C. triramosus* and a male cyathostome in Figures 4–8.

Various filamentous organisms inhabit mammalian guts including mice, rats, swine, and humans (Savage, 1983a, b). However, the cyathostomid nematode–filamentous bacterial association discussed here is, as far as can be determined, the first report of its kind in a mammalian host. This is the first attempt to quantify this association.

The predominance of female cyathostomes affected as compared to that of male cyathostomes is significant ($P < 0.001$), and the colonization of the vulvar orifices in this population is evident in this study. The numbers of each species (Fig. 1) of female cyathostome were compared with and without bacteria and differed significantly from one another ($P < 0.001$). There were, however, no significant differences among species of male cyathostomes (Fig. 2) for a similar comparison. Numbers of female cyathostomes were compared for each species with the association of bacteria at the vulva and at the anus, and in all cases there was a significant difference ($P \leq 0.031$).

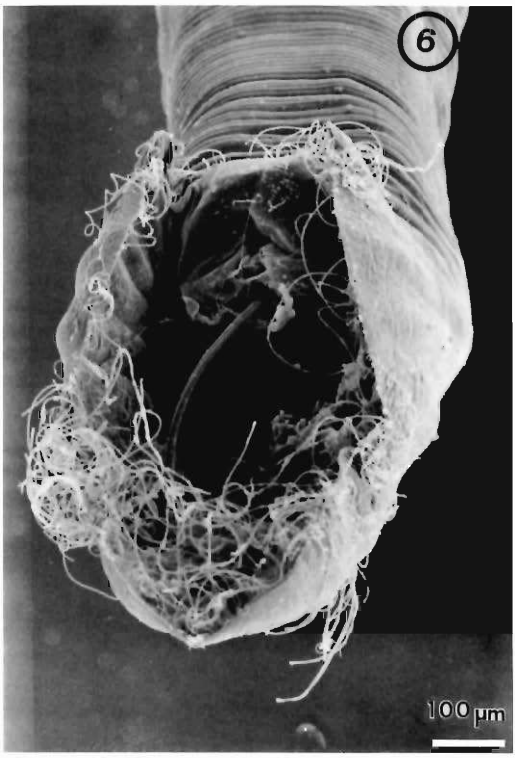


Figure 6. Ventral surface of dorsal ray of copulatory bursa of male cyathostome. Presence of filamentous bacteria suggests transmission during copulation.

Few adult male cyathostomes demonstrate the association, and only the ventral side of the copulatory bursa of the males is affected. Because this ventral side would clasp the female cyathostome during copulation, it is possible that the small numbers of males become affected at this time. It is possible that only reproductively successful males are colonized by the bacteria and these males infected all of the females.

When males are affected, it is always a smaller proportion than in females of the same species. In *C. triramosus,* the second most abundant species, most females (110 out of 112) were affected. Although the vulva is the orifice predominantly affected throughout, the anus of a small number of *C. montgomeryi* was sometimes colonized. Earlier studies suggested that the colonization of

Figures 7, 8. ***Cylicocyclus triramosus*** **female with dense mass of filamentous bacteria on the posterior extremity. 7. Light microscopy shows mass of microbes near vulva (arrow). 8. Scanning electron microscopy showing mass of microbes.**

the anus by filamentous bacteria was caused by the release of an excretory product that provided a suitable environment (Mackie et al., 1989). However, the present study indicates that the vulva, and not the anus, is the colonized site. Perhaps some substance in the female reproductive tract serves as a suitable substrate for colonization and, consequently, this association may influence egg production.

Uterine tracts of the cyathostomes in the present study were examined for the presence of eggs. Examination for the penetration of the microbes into the reproductive tract canal was inconclusive in some species, such as in *C. triramosus,* because the dense mass of microbes (Fig. 7) prevented thorough examination using light microscopy. In future studies, the penetration of these bacteria in the reproductive and digestive tracts could be examined with the use of differential staining and serial sectioning of the uterine tract of these females.

Observations such as those in the present study are urged by the difficulty to cultivate these filamentous bacteria. Though attempts have been made (Krecek et al., 1992), this inability hampers possible studies on the dynamics of this association.

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Research Note

Sarcocystis felis (Protozoa: Sarcocystidae) from the African Lion (*Panthera leo*)

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ABSTRACT: Sarcocysts of *Sarcocystis felis* were found in skeletal muscle of a 7-yr-old African lioness (*Panthera leo*) from Kenya, Africa. Sarcocysts were up to 1,650 μm long and up to 150 μm wide. The cyst wall was 1.0–1.5 μm thick and had characteristic fingerlike villar projections.

KEY WORDS: *Sarcocystis felis*, lion, *Panthera leo*, Sarcocysts.

Sarcocystis spp. undergo a 2-host life cycle involving prey and predator animals. Infection of muscles by *Sarcocystis* spp. (sarcocysts) is common in herbivores. Until recently, infection of muscles by sarcocysts in carnivores was considered rare (Dubey et al., 1989). Sarcocysts were found in more than half of bobcats, cougars, and panthers examined in 3 studies (Greiner et al., 1989; Anderson et al., 1992; Dubey et al., 1992). Only one morphologic type of sarcocyst was found in bobcats (*Felis rufus*), domestic cats (*Felis domesticus*), Florida panthers (*Felis concolor coryi*), and cougars (*Felis concolor floridanus*). Dubey et al. (1992) proposed the name *Sarcocystis felis* for *Sarcocystis* in muscles of Felidae. Although sarcocysts were reported previously from lions from India (Bhatavedkar and Purohit, 1963; Somvanshi et al., 1987) and Africa (Bwangamoi et al., 1990), the purpose of this article is to report species of *Sarcocystis* from the African lion, *Panthera leo* (*Felis leo*).

Specimens of heart and skeletal muscle from a 7-yr-old lioness from Nairobi National Park, Kenya, were fixed in 10% formalin solution (Bwangamoi et al., 1990). The lioness was euthanized because of rabies virus infection (Bwangamoi et al., 1990). Paraffin sections were cut at 5–6 μm thickness and examined microscopically after staining with hematoxylin and eosin.

Sarcocysts were seen in 11 of 15 sections of